PHTHALATES, PARABENS, AND BISPHENOL A: "NON-PERSISTENT BUT PERSISTENTLY PRESENT" COMPOUNDS

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Introduction

The safety of food and personal care products is a main priority for food and cosmetic industries and for consumers' health protection. Both types of products may contain hundreds of substances with a high potential for human exposure. Consequently an increasingly pressing demand to know and control their composition in depth exists. Among the exogenous chemical compounds contained in food and personal care products, phthalates (phthalic acid esters, PAEs), parabens, and bisphenol A (BPA) have received special attention in the last years due to the clear evidences of their reproductive toxicity[1,2], their estrogenic activity[3-6], and their ubiquitous presence in the environment[7-10], being possible to consider them as "non-persistent but persistently present" compounds. PAEs are commonly used as solvents and odorless diluents in cosmetic products such as deodorants, hair products and perfumes[11] and, since they are plasticizers, they are also found in the food industry as food wraps[12]. Parabens are a group of synthetic chemicals that are widely used as preservatives in the cosmetic, pharmaceutical and food industries, due to their bactericidal and fungicidal properties and their effectiveness and low cost. Similarly to PAEs, BPA is intensively employed as a monomer in the production of polycarbonates and epoxy resins, used for food containers[12].

Since PAEs, parabens, and BPA are extensively used in consumer products, a reliable analytical method is needed to monitor and quantify these compounds. Some analytical methods have previously been reported for the determination of PAEs, BPA and parabens[13-16]. However, analytical methods designed to simultaneously determine as many ingredients as possible with minimal effort would be welcome for both the industry and the control bodies worldwide. In this study, a multiclass method based on gas chromatography coupled to a triple quadrupole mass spectrometer analyzer working in multiple reactions monitoring mode (GC-QqQ(MRM)) for the rapid and simultaneous determination of thirteen "non-persistent but persistently present" compounds (five PAEs, seven parabens, and BPA) was developed.

Materials and methods

Native standards of dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), diethyl hexyl phthalate (DEHP), methyl paraben (MP), ethyl paraben (EP), *n*-propyl paraben (nPP), *iso*-propyl paraben (iPP), *n*-butyl paraben (nBP), *iso*-butyl paraben (iPB), benzyl paraben (BzP), and bisphenol A (BPA) were supplied by AccuStandard (New Haven, CT, USA). The isotopically labeled standards DMP-D_4, DEP-D_4, DBP-D_4, BBP-D_4, DEHP-D_4, MP-$^{13}C_6$, nBP-$^{13}C_6$, BPA-$^{13}C_{12}$ were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). (Trimethylsilyl)diazomethane (TMSDM, 2.0 M in hexane), acetic anhydre, N-O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchloro silane (TMCS), and pyridine were purchased from Sigma-Aldrich (St Louis, MO, USA).

For the in-solution derivatization, adequate volume of each working standard solution was evaporated to dryness under nitrogen at 50 ºC. The residue was derivatized by adding 50 µL of the silylating agent, containing BSTFA and 1% TMCS as catalyst, and 50 µL of pyridine according to De Luca *et al.*[17]. The vial was vortex and heated at 80 ºC for 30 min. After cooling, the derivatized solution was evaporated to dryness, and afterwards, re-dissolved in the adequate volume of isooctane before analysis.

PAEs, parabens, and BPA were determined by using a GC-QqQ system consisting of a Trace GC Ultra gas chromatograph coupled with a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Sci., San José, CA, USA) equipped with an electron ionization (EI) source. A GC capillary column Factor Four VF-5HT (5% phenyl methyl polysiloxane, 15m, 0.25 mm i.d., 0.1 µm film thickness) purchased from Agilent Technologies (Palo Alto, CA, USA) was used for separation. Standards and samples were injected in a PTV hot splitless mode programmed at 90 ºC hold for 0.05 min then raise at 14.5 ºC s^{-1} to 280 ºC (maintained 5 min) and then raise at 14.5 ºC s^{-1} to 300 ºC (maintained 5 min). The splitless time was set to 1.5 min. The column temperature was programmed as follows: 90 °C was maintained for 1.5 min, then the temperature was increased at 5 °C min^{-1} to 280 °C (maintained 1 min), afterwards at 10 °C min^{-1} to 300 ºC and held for 5 min. Helium was used as the

carrier gas at a constant flow rate of 1.0 mL min^{-1}. The temperature of the transfer line and the ion source were set at 300 °C and 240 ºC, respectively. Analysis was carried out in the multiple reaction monitoring (MRM), using two specific combinations of a precursor-product ion transition for each compound.
Due to the ubiquitous nature of PAEs and to minimize the contamination, all glassware was washed and gently shacked with acetone, dichlorometane, hexane, and methanol, and next muffled at 400 °C during 6 h prior to use. Plastic materials that could contain PAEs were avoided during the whole sample treatment.

Results and discussion.

Derivatization of parabens and BPA

The analysis of PAEs has been widely performed by GC. However, parabens and BPA are less volatile, and require to be derivatized before the analysis by GC. Four different methods for the derivatization of parabens and BPA in solution were explored, following recommended published methods for similar derivative reactions: TMSDM[18], acetic anhydride and pyridine[19], BSTFA and TMCS (99:1, v/v)[20], and BSTFA/TMCS (99:1 v/v)[17] in presence of pyridine. All four derivatization methods were experimentally tested and compared.
The derivatization of parabens with TMSDM resulted in the apparition of three different peaks identified by the NIST as the ethyl derivate, the non-derivatized compound, and the trimethylsilyl species, respectively. As an example, Figure 1 shows the chromatogram (acquired in SCAN mode) and the spectra corresponding to the three peaks that appear for MP.

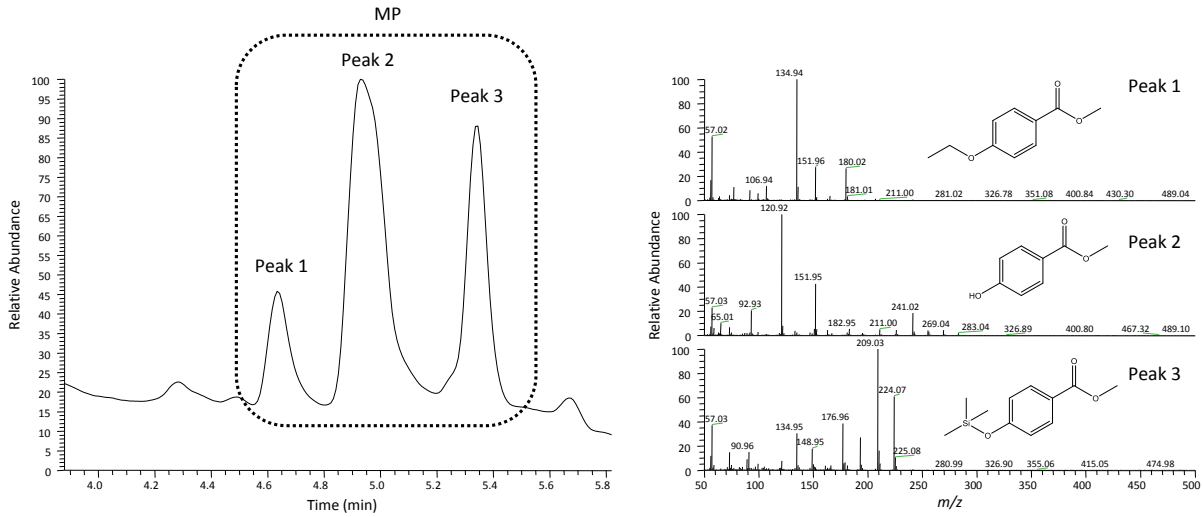

Figure 1. Chromatogram and spectra of the derivatized MP using TMSDM as derivatization agent.

The derivatization of parabens with acetic anhydride in presence of pyridine has demonstrated to be efficient previously[19]. However, even following the same experimental conditions as the reported in the cited work, in our case the derivatized parabens gave broad tailing peaks. On the other hand, the derivatization with BSTFA/TMCS both in presence and absence of pyridine resulted in an efficient trimethylsilylation of both parabens and BPA. However, in presence of pyridine the peaks corresponding to parabens were sharped. For this reason, derivatization of parabens and BPA with BSTFA/TMCS/pyridine was chosen as the optimum derivatization method and used afterwards. In addition, the derivatization procedure was tested in presence of PAEs in order to check any possible reaction with these compounds, and no variation on the PAE response was observed before and after the derivatization step.

GC-QqQ(MRM) instrumental method

A GC-QqQ(MRM) method for the simultaneous determination of five PAEs, seven parabens, and BPA has been developed. For this purpose, the most abundant ions of the SCAN spectra produced by electron ionization (EI) for each congener at 40 eV and an emission current of 50 mA were selected. A lower eV than usual (70 eV) was fixed in the present method in order to reduce de high fragmentation observed for PAEs in EI sources. Even in these conditions, no molecular ion was obtained for DMP and very small signal of the molecular ions corresponding to the rest of PAEs was observed, being no possible to use the molecular ion as precursor ion. The most abundant ion corresponds to fragment 149 *m/z*, for DEP, DBP and, BBP; to 163 *m/z* for DMP; and to 167 *m/z* for DEHP. In the case of parabens, trimethylsilylated molecular ion was one of the most abundant ions for all the parabens investigated, except iPP, and could be selected as precursor ion for the quantitation transition. The same happened for BPA, being one of the most abundant transition that corresponding to the loose of CH_3 group (15 *m/z*) from the trimethylsilylated molecular ion (372 *m/z*). The confirming transitions were chosen in all cases considering the maximum sensitivity (highest response) and, as much as possible, maximum selectivity (selecting characteristic ions for each compound).
Precursor-product transitions along with their corresponding optimized collision energies are shown in Table 1. As it has been previously mentioned, some of the transitions selected for PAEs are the same for the different compounds making the MRM method less selective than those of parabens and BPA.

Table 1. MRM transitions, at their corresponding collision energies, selected for native PAEs, parabens, and BPA.

Compound	MRM (*m/z*)	Collision energy (eV)	Compound	MRM (*m/z*)	Collision energy (eV)
DMP	163 > 77	18	MP	224 > 209	10
	194 > 163	10		209 > 177	8
DEP	149 > 65	18	EP	238 > 223	8
	177 > 149	9		223 > 151	9
DBP	149 > 65	19	iPP	210 > 195	8
	223 > 149	12		237 > 221	6
BBP	149 > 65	19	nPP	210 > 195	8
	206 > 149	10		252 > 195	14
DEHP	167 > 149	8	iBP	210 > 195	9
	279 > 149	14		266 > 210	8
BPA	357 > 73	27	nBP	210 > 195	10
	372 > 357	13		266 > 210	7
			BzP	300 > 193	22
				285 > 91	14

Under these experimental conditions, the chromatogram obtained for the multiclass separation of five PAEs, seven parabens, and BPA was that gathered in Figure 2.

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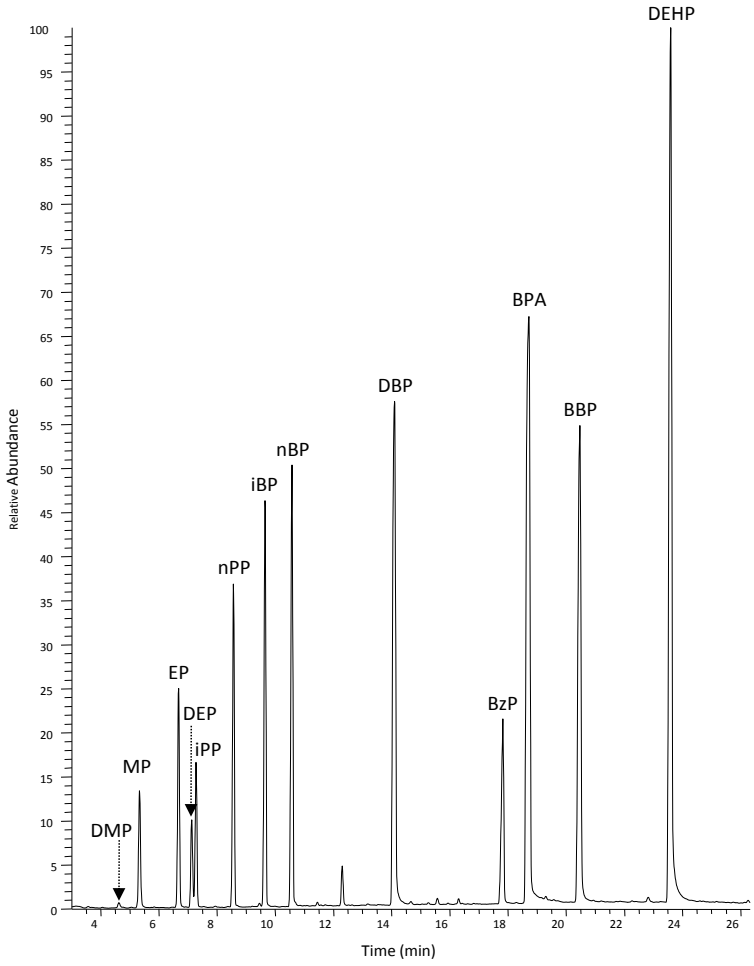

Figure 2. Chromatogram corresponding to the multiclass separation of five PAEs, seven parabens, and BPA.